

CLAIMS:

1. A gene cassette comprising a combination of genes which, in an appropriate strain background, are able to direct the synthesis of mycaminose or angolosamine and to direct its subsequent transfer to an aglycone or pseudoaglycone.

2. A gene cassette according to claim 1, comprising a combination of genes able to direct the synthesis and transfer of mycaminose, wherein:

- a) at least one of the genes is selected from the group consisting of: *angorf14*, *tylMIII*, *tylMI*, *tylB*, *tylAI*, *tylAII*, *tylIa*, *angAI*, *angAII*, *angMIII*, *angB*, *angMI*, *eryG* and *eryK*; and,
- b) at least one of the genes is a glycosyltransferase gene selected from the group consisting of *tylMII*, *angMII*, *desVII*, *eryCIII*, *eryBV*, *spnP*, and *midI*.

3. A gene cassette according to claim 2, wherein one of the genes within the gene cassette is *tylIa*

4. A gene cassette according to claim 2, wherein one of the genes within the gene cassette is *angorf14*

5. A gene cassette according to claim 2 or 4, which comprises *angAI*, *angAII*, *angorf14*, *angMIII*, *angB* and *angMI*, in combination with one or more glycosyltransferase genes selected from the group consisting of *eryCIII*, *tylMII* and *angMII*.

6. A gene cassette according to claim 2 or 3, which comprises *tylAI*, *tylAII*, *tylMIII*, *tylB*, *tylIa* and *tylMI*, in combination with one or more glycosyltransferase genes selected from the group consisting of *eryCIII*, *tylMII* and *angMII*.

7. A gene cassette according to claim 1 comprising a combination of genes able to direct the synthesis and transfer of angolosamine, wherein:

- a) at least one of the genes is selected from the group consisting of: *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf14*, *angorf4*, *tylMIII*, *tylMI*, *tylB*, *tylAI*, *tylAII*, *eryCVI*, *spnO*, *eryBVI*, and *eryK*; and,
- b) at least one of the genes is a glycosyltransferase gene selected from the group consisting of *eryCIII*, *tylMII*, *angMII*, *desVII*, *eryBV*, *spnP* and *midI*.

8. A gene cassette according to claim 7, which comprises *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf14* and *spnO*, in combination with one or more glycosyltransferase genes selected from the group consisting of *angMII*, *tylMII* and *eryCIII*.

9. A gene cassette according to claim 7, which comprises *angMIII*, *angMI*, *angB*, *angAI*, *angAII*, *angorf4*, and *angorf14*, in combination with one or more glycosyltransferase genes selected from the group consisting of *angMII*, *tylMII* and *eryCIII*.

10. A process for the production of erythromycins and azithromycins which contain either mycaminose or angolosamine at the C-5 position, said process comprising transforming a strain with a gene cassette as described in any one of claims 1-9 above and culturing the strain under appropriate conditions for the production of said erythromycin or azithromycin.

11. The process of claim 10, wherein the strain is selected from actinomycetes, *Pseudomonas*, myxobacteria, and *E. coli*.

12. The process of claim 10, wherein the host strain is additionally transformed with the *ermE* from *S. erythraea*.

13. The process of claim 10 or claim 11, wherein the host strain is an actinomycete.

14. The process of claim 13, wherein the host strain is selected from *S. erythraea*, *Streptomyces griseofuscus*, *Streptomyces cinnamonensis*, *Streptomyces albus*, *Streptomyces lividans*, *Streptomyces hygroscopicus* sp., *Streptomyces hygroscopicus* var. *ascomyceticus*, *Streptomyces longisporoflavus*, *Saccharopolyspora spinosa*, *Streptomyces tsukubaensis*, *Streptomyces coelicolor*, *Streptomyces fradiae*, *Streptomyces rimosus*, *Streptomyces avermitilis*, *Streptomyces eurythermus*, *Streptomyces venezuelae*, and *Amycolatopsis mediterranei*.

15. The process of claim 14, wherein the host strain is *S. erythraea*.

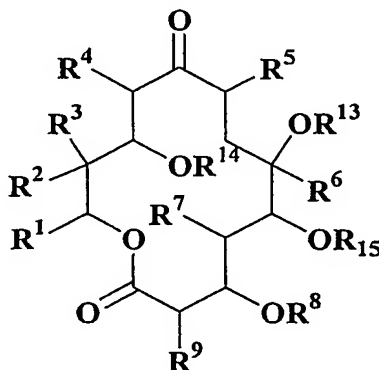
16. The process of claim 15, wherein the host strain is selected from the SGQ2, Q42/1 or 18A1 strains of *S. erythraea*.

17. The process of any one of claims 10 to 16, which further comprises feeding of an aglycone and/or a pseudoaglycone substrate to the recombinant strain.

18. The process of claim 17, wherein said aglycone and/or pseudoaglycone is selected from the group consisting of 3-*O*-mycarosyl erythronolide B, erythronolide B, 6-deoxy erythronolide B, 3-*O*-mycarosyl-6-deoxy erythronolide B, tylactone, spinosyn pseudoaglycone, 3-*O*-rhamnosyl erythronolide B, 3-*O*-
 5 rhamnosyl-6-deoxy erythronolide B, 3-*O*-angolosaminyl erythronolide B, 15-hydroxy-3-*O*-mycarosyl erythronolide B, 15-hydroxy erythronolide B, 15-hydroxy-6-deoxy erythronolide B, 15-hydroxy-3-*O*-mycarosyl-6-deoxy erythronolide B, 15-hydroxy-3-*O*-rhamnosyl erythronolide B, 15-hydroxy-3-*O*-rhamnosyl-6-deoxy erythronolide B, 15-hydroxy-3-*O*-angolosaminyl erythronolide B, 14-hydroxy-3-*O*-mycarosyl erythronolide B, 14-hydroxy erythronolide B, 14-hydroxy-6-deoxy erythronolide B, 14-hydroxy-
 10 3-*O*-mycarosyl-6-deoxy erythronolide B, 14-hydroxy-3-*O*-rhamnosyl erythronolide B, 14-hydroxy-3-*O*-rhamnosyl-6-deoxy erythronolide B, 14-hydroxy-3-*O*-angolosaminyl erythronolide B.

19. The process of any one of claims 10 to 18, which additionally comprises the step of isolating the compound produced.

20. A compound according to the formula I below:



R^1 is selected from:

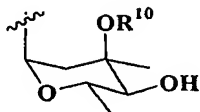
- H, CH₃, C₂H₅
- an alpha-branched C₃-C₈ group selected from alkyl, alkenyl, alkynyl, alkoxyalkyl and alkylthioalkyl groups any of which may be optionally substituted by one or more hydroxyl groups;
- a C₅-C₈ cycloalkylalkyl group wherein the alkyl group is an alpha-branched C₂-C₅ alkyl group
- a C₃-C₈ cycloalkyl group or C₅-C₈ cycloalkenyl group, either of which may optionally be substituted by one or more hydroxyl, or one or more C₁-C₄ alkyl groups or halo atoms

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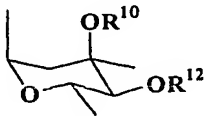
- a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups, halo atoms or hydroxyl groups
- phenyl which may be optionally substituted with at least one substituent selected from C₁-C₄ alkyl, C₁-C₄ alkoxy and C₁-C₄ alkylthio groups, halogen atoms, trifluoromethyl, and cyano or
- R¹⁷-CH₂- where R¹⁷ is H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, alkoxyalkyl or alkylthioalkyl containing from 1 to 6 carbon atoms in each alkyl or alkoxy group wherein any of said alkyl, alkoxy, alkenyl or alkynyl groups may be substituted by one or more hydroxyl groups or by one or more halo atoms; or a C₃-C₈ cycloalkyl or C₅-C₈ cycloalkenyl either of which may be optionally substituted by one or more C₁-C₄ alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups or halo atoms; or a group of the formula SA₁₆ wherein A₁₆ is C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₅-C₈ cycloalkenyl, phenyl or substituted phenyl wherein the substituent is C₁-C₄ alkyl, C₁-C₄ alkoxy or halo, or a 3 to 6 membered oxygen or sulphur-containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups or halo atoms

R², R⁴, R⁵, R⁶, R⁷ and R⁹ are each independently H, OH, CH₃, C₂H₅ or OCH₃

R³= H or OH

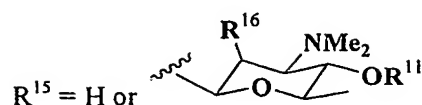
R⁸ = H, , rhamnose, 2'-O-methyl rhamnose, 2',3'-bis-O-methyl rhamnose, 2',3',4'-tri-O-methyl rhamnose, oleandrose, olioside, digitoxose, olivose or angolosamine;

R¹⁰= H or CH₃ or C(=O)R_A, where R_A = C₁-C₆ alkyl, C₂-C₆ alkenyl or C₂-C₆ alkynyl

R¹¹ = H, , mycarose, C4-O-acyl-mycarose or glucose

R¹²= H or C(=O)R_A, where R_A = C₁-C₆ alkyl, C₂-C₆ alkenyl or C₂-C₆ alkynyl

R¹³= H or CH₃



$R^{15} = H$ or

$R^{16} = H$ or OH

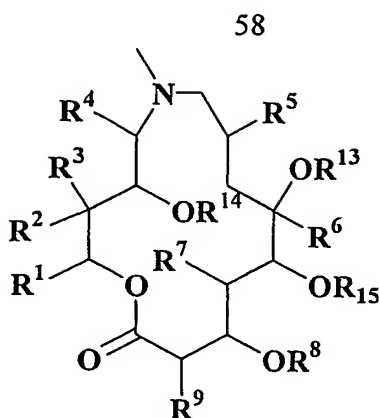
$R^{14} = H$ or $-C(O)NR^eR^d$ wherein each of R^e and R^d is independently H, C_1 - C_{10} alkyl, C_2 - C_{20} alkenyl, C_2 - C_{10} alkynyl, $-(CH_2)_m(C_6$ - C_{10} aryl), or $-(CH_2)_m$ (5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein each of the foregoing R^e and R^d groups, except H, may be substituted by 1 to 3 Q groups; or wherein R^e and R^d may be taken together to form a 4-7 membered saturated ring or a 5-10 membered heteroaryl ring, wherein said saturated and heteroaryl rings may include 1 or 2 heteroatoms selected from O, S and N, in addition to the nitrogen to which R^e and R^d are attached, and said saturated ring may include 1 or 2 carbon-carbon double or triple bonds, and said saturated and heteroaryl rings may be substituted by 1 to 3 Q groups; or R^2 and R^{17} taken together form a carbonate ring; each Q is independently selected from halo, cyano, nitro, trifluoromethyl, azido, $-C(O)Q^1$, $-OC(O)Q^1$, $-C(O)OQ^1$, $-OC(O)OQ^1$, $-NQ^2C(O)Q^3$, $-C(O)NQ^2Q^3$, $-NQ^2Q^3$, hydroxy, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, $-(CH_2)_m(C_6$ - C_{10} aryl), and $-(CH_2)_m$ (5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein said aryl and heteroaryl substituents may be substituted by 1 or 2 substituents independently selected from halo, cyano, nitro, trifluoromethyl, azido, $-C(O)Q^1$, $-C(O)OQ^1$, $-OC(O)OQ^1$, $-NQ^2C(O)Q^3$, $-C(O)NQ^2Q^3$, $-NQ^2Q^3$, hydroxy, C_1 - C_6 alkyl, and C_1 - C_6 alkoxy;

each Q^1 , Q^2 and Q^3 is independently selected from H, OH, C_1 - C_{10} alkyl, C_1 - C_6 alkoxy, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, $-(CH_2)_m(C_6$ - C_{10} aryl), and $-(CH_2)_m$ (5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4; with the proviso that the compound is not 5-O-dedesosaminy-5-O-mycaminy-erythromycin A or D

or said compound is a variant of any of the above in which the $-CHOR^{14}$ - at C11 is replaced by a methylene group ($-CH_2-$), a keto group ($C=O$), or by a 10,11-olefinic bond;

or said compound is a variant of any of the above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: $-CO-$, $-CH(OH)-$, alkene $-CH-$, and CH_2); with the proviso that the compounds are not selected from the group consisting of 5-O-dedesosaminy-5-O-mycaminy-erythromycin A and 5-O-dedesosaminy-5-O-mycaminy-erythromycin D.

21. A compound according to the formula II below:



R¹ is selected from:

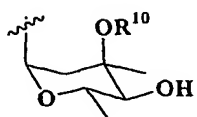
- H, CH₃, C₂H₅
- an alpha-branched C₃-C₈ group selected from alkyl, alkenyl, alkynyl, alkoxyalkyl and alkylthioalkyl groups any of which may be optionally substituted by one or more hydroxyl groups;
- a C₅-C₈ cycloalkylalkyl group wherein the alkyl group is an alpha-branched C₂-C₅ alkyl group
- a C₃-C₈ cycloalkyl group or C₅-C₈ cycloalkenyl group, either of which may optionally be substituted by one or more hydroxyl, or one or more C₁-C₄ alkyl groups or halo atoms
- a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups, halo atoms or hydroxyl groups
- phenyl which may be optionally substituted with at least one substituent selected from C₁-C₄ alkyl, C₁-C₄ alkoxy and C₁-C₄ alkylthio groups, halogen atoms, trifluoromethyl, and cyano or
- R¹⁷-CH₂- where R¹⁷ is H, C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, alkoxyalkyl or alkylthioalkyl containing from 1 to 6 carbon atoms in each alkyl or alkoxy group wherein any of said alkyl, alkoxy, alkenyl or alkynyl groups may be substituted by one or more hydroxyl groups or by one or more halo-atoms; or a C₃-C₈ cycloalkyl or C₅-C₈ cycloalkenyl either of which may be optionally substituted by one or more C₁-C₄ alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups or halo atoms; or a group of the formula SA₁₆ wherein A₁₆ is C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, C₃-C₈ cycloalkyl, C₅-C₈ cycloalkenyl, phenyl or substituted phenyl wherein the substituent is C₁-C₄ alkyl, C₁-C₄ alkoxy or halo, or a 3 to 6 membered oxygen or sulphur-containing heterocyclic ring

which may be saturated, or fully or partially unsaturated and which may optionally be substituted by one or more C₁-C₄ alkyl groups or halo atoms

R², R⁴, R⁵, R⁶, R⁷ and R⁹ are each independently H, OH, CH₃, C₂H₅ or OCH₃

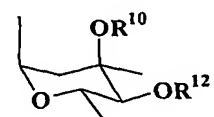
R³ = H or OH

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 R⁸ = H, , rhamnose, 2'-O-methyl rhamnose, 2',3'-bis-O-methyl rhamnose, 2',3',4'-tri-O-methyl rhamnose, oleandrose, olioside, digitoxose, olivose or angolosamine;

R¹⁰ = H or CH₃ or C(=O)R_A, where R_A = C1-C6 alkyl, C2-C6 alkenyl or C2-C6 alkynyl

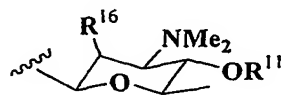
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 R¹¹ = H, , mycarose, C4-O-acyl-mycarose or glucose

R¹² = H or C(=O)R_A, where R_A = C1-C6 alkyl, C2-C6 alkenyl or C2-C6 alkynyl

R¹³ = H or CH₃

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 R¹⁵ = H or

R¹⁶ = H or OH

R¹⁴ = H or -C(O)NR^cR^d wherein each of R^c and R^d is independently H, C₁-C₁₀ alkyl, C₂-C₂₀ alkenyl, C₂-C₁₀ alkynyl, -(CH₂)_m(C₆-C₁₀ aryl), or -(CH₂)_m(5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein each of the foregoing R^c and R^d groups, except H, may be substituted by

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1 to 3 Q groups; or wherein R^c and R^d may be taken together to form a 4-7 membered saturated ring or a 5-10 membered heteroaryl ring, wherein said saturated and heteroaryl rings may include 1 or 2

heteroatoms selected from O, S and N, in addition to the nitrogen to which R^c and R^d are attached, and said saturated ring may include 1 or 2 carbon-carbon double or triple bonds, and said saturated and

heteroaryl rings may be substituted by 1 to 3 Q groups; or R² and R¹⁷ taken together form a carbonate

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ring; each Q is independently selected from halo, cyano, nitro, trifluoromethyl, azido, -C(O)Q¹, -OC(O)Q¹, -C(O)OQ¹, -OC(O)OQ¹, -NQ²C(O)Q³, -C(O)NQ²Q³, -NQ²Q³, hydroxy, C₁-C₆ alkyl, C₁-C₆ alkoxy, -(CH₂)_m(C₆-C₁₀ aryl), and -(CH₂)_m(5-10 membered heteroaryl), wherein m is an integer ranging from 0 to 4, and wherein said aryl and heteroaryl substituents may be substituted by 1 or 2 substituents independently selected from halo, cyano, nitro, trifluoromethyl, azido, -C(O)Q¹, -C(O)OQ¹, -OC(O)OQ¹, -NQ²C(O)Q³, -C(O)NQ²Q³, -NQ²Q³, hydroxy, C₁-C₆ alkyl, and C₁-C₆ alkoxy;

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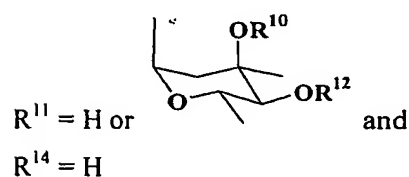
each Q^1 , Q^2 and Q^3 is independently selected from H, OH, C_1 - C_{10} alkyl, C_1 - C_6 alkoxy, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, $-(CH_2)_m(C_6-C_{10} \text{ aryl})$, and $-(CH_2)_m(5-10 \text{ membered heteroaryl})$, wherein m is an integer ranging from 0 to 4;

or said compound is a variant of any of the above in which the $-CHOR^{14}-$ at C12 is replaced by a methylene group ($-CH_2-$), a keto group ($C=O$), or by a 11,12-olefinic bond;

or said compound is a variant of any of the above which differs in the oxidation state of one or more of the ketide units (i.e. selection of alternatives from the group: $-CO-$, $-CH(OH)-$, alkene $-CH-$, and CH_2).

22. A compound according to claim 20 or 21, wherein: R^2 , R^4 , R^5 , R^6 , R^7 and R^9 are all CH_3

23. A compound according to claim 22, wherein



24. A compound according to claim 23, wherein $R^1 = C_2H_5$ optionally substituted with a hydroxyl group

25. A compound according to claim 24, wherein $R^{12} = H$

26. A compound according to claim 25, wherein $R^1 = C_2H_5$